

Claims:

1. A method for development of nucleotide probes for myctophid fishes, said method comprising the steps of :
 - (i) extracting the DNA from the muscle tissue of a myctophid fish,
 - (ii) selecting gene regions in the extracted DNA with the selected primers and the amplifying the same using polymerase chain reaction (PCR),
 - (iii) eluting the PCR amplified DNA,
 - (iv) reamplifying the gene regions from PCR amplified DNA and eluting the same,
 - (v) cycle sequencing of eluted DNA using a single primer,
 - (vi) purifying extension products,
 - (vii) sequencing the extension product on acrylamide gel,
 - (viii) confirming the sequences for the target gene by Blast -Email,
 - (ix) ligating the eluted PCR products in a vector,
 - (x) preparing the electro-competent cells for electro transformation,
 - (xi) electro transforming the host cells,
 - (xii) growing and harvesting of transformed host cells,
 - (xiv) confirming that the transformed bacteria has the plasmids with the gene inserts by PCR.
 - (xv) purifying recombinant plasmid DNA having the cloned gene probes from the transformed host cells,
 - (xvi) checking purity and specificity of the cloned DNA probe insert by cutting with restriction enzyme,
 - (xvii) confirming the molecular size of the DNA probe insert,
 - (xviii) PCR amplification of the gene insert from the probe using both primers,
 - (xix) eluting of the amplified gene region,
 - (xx) cycle sequencing of the gene region of the probe,
 - (xxi) sequencing of the cloned DNA insert on acrylamide gel,
 - (xxii) comparing the DNA sequence of the prepared DNA probes using "BLAST program "against the known sequences of similar genes in the genome data bases,

- (xxiii) confirming the sequences of the cloned probe by aligning with sequences of the claim 1(vii), and
- (xxiv) designing species specific primers from the sequences.
2. A method as claimed in claim 1 wherein the myctophid fishes are selected from the group comprising *Stenobrachis leucopsarus*, *Diaphus theta*, *Protomyctophum crockeri*, *Tarletonbeania crenularis* and *Lampanyctus regalis*.
 3. A method as claimed in claim 1 wherein the gene regions are selected from mitochondrial and nuclear genes.
 4. A method claimed in claim 1 wherein the mitochondrial genes taken for probe preparation are selected from the group comprising: Cyt b and D-loop genes, 12 S RNA and 16 S RNA genes.
 5. A method claimed in claim 1 wherein the nuclear genes taken for probe preparation are selected from Rod and ITS-2 genes.
 6. A method of claim 1 wherein the PCR amplified cleaned nuclear gene probe is Rod gene.
 7. A method claimed in claim 1 wherein the nuclear gene taken for the cloned probe preparation is ITS-2 gene.
 8. A method as claimed in claim 1 wherein the concentration of primers used for PCR amplification is 20 μ eu. L.
 9. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for amplification and detection of Cyt b gene contains oligonucleotides with the sequences:
 CYT 1: 5' TGA YTT GAA RAA CCA YCG TTG 3'
 CYT 2: 5' CTC CAR TCT TCG RYT TAC AAG 3'
 10. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for reamplification and detection of Cyt b gene contains oligonucleotides with the sequences:
 CBI-L: 5' CCA TCC AAC ATC TCA GCA TGA TGA AA 3'
 CYT 2: 5' CTC CAR TCT TCG RYT TAC AAG 3'

11. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification and detection of D-Loop gene contains oligonucleotides with the sequences :
PRO-L : 5' CTA CC 3'
D-LOOP H: 5' CCT GAA GTA GGA ACC AGA TG 3'
12. A method as claimed in claim 1 wherein the forward and backward primers used for PCR amplification of ITS2 gene were
ITS1 F : 5' TTG TAC ACA CCGCCCGTC GC 3'
ITS2 R: 5' ATA TGC TTA AAT TCA GCG GG 3'
13. A method as claimed in claim 1 wherein the forward and backward primers used for PCR reamplification of ITS2 gene from ITS1 F and ITS2 R PCR amplification were
ITS2 F: 5' CTA CGC CTG TCT GAG TGT C 3'
ITS2 R: 5' ATA TGC TTA AAT TCA GCG GG 3'
14. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of Rhodopsin gene Rod contains oligonucleotides with the sequences:
ROD-F: 5' CAT ATG AAT ACC CTC AGT ACT ACC 3'
ROD-R: 5' TCT TTC CGC AGC ACA ACG TGG 3'
15. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of 12S RNA gene contains oligonucleotides with the sequences:
12 SA-L: 5' AAA CTG GGA TTA GAT ACC CCA CTA T 3'
12 SB-H: 5' AGA GTG ACG GGC GGT GTG T 3'
16. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of 16S RNA gene contains oligonucleotides with the sequences:
16 SAR -L: 5' CGC CTG TTT ATC AAA AAC AT 3'
16 SBR-H: 5' CCG GTC TGA ACT CAG ATC ACG T 3'
17. A method as claimed in claim 1 wherein the forward and backward primers used for PCR amplification of Rhodopsin gene Rod were :

- ROD-F: 5' CAT ATG AAT ACC CTC AGT ACT ACC 3'
- ROD-R: 5' TCT TTC CGC AGC ACA ACG TGG 3'
18. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of 12S RNA gene were :
- 12 SA-L: 5' AAA CTG GGA TTA GAT ACC CCA CTA T 3'
- 12 SB-H: 5' AGA GTG ACG GGC GGT GTG T 3'
19. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of 16S RNA gene were :
- 16 SAR -L: 5' CGC CTG TTT ATC AAA AAC AT 3'
- SBR-H: 5' CCG GTC TGA ACT CAG ATC ACG T 3'
20. A method claimed in claim 1 wherein the 12S RNA gene and 16S RNA gene in the myctophid fish *Stenobrachius leucopsarus* were amplified by PCR.
21. A method claimed in claim 1 wherein the 12S RNA and 16S RNA gene in myctophid fish *Diaphus theta* were eluted by PCR amplification.
22. A method claimed in claim 1 wherein the elution of PCR amplification products of myctophid fish *Protomyctophum crockeri*, resulted in 12 S RNA.
23. A method claimed in claim 1 wherein the elution of PCR amplification products of myctophid fish *Protomyctophum crockeri*, resulted in 16 S RNA.
24. A method claimed in claim 1 wherein the elution of PCR amplification of myctophid fish *Tarletonbeania crenularis*, resulted in 12 S RNA.
25. A method claimed in claim 1 wherein the elution of PCR amplification of myctophid fish *Tarletonbeania crenularis*, resulted in 16 S RNA.
26. A method claimed in claim 1 wherein the elution of PCR amplification of myctophid fish *Lampanyctus regalis*, resulted in 12 S RNA.
27. A method claimed in claim 1 wherein the elution of PCR amplification of myctophid fish *Lampanyctus regalis*, resulted in 16 S RNA.
28. A method claimed in claim 1 wherein the cycle sequencing primer concentration used was 2 μ L,
29. A method claimed in claim 1 wherein the cycle sequencing primer for CYT b gene consisted of oligonucleotides with the sequence:
- CYT 1: 5' TGA YTT GAA RAA CCA YCG TTG 3'

30. A method claimed in claim 1 wherein the cycle sequencing primer for CYT b gene consisted of oligonucleotides with the sequence:
CYT 2: 5' CTC CAR TCT TCG RYT TAC AAG 3'
31. A method claimed in claim 1 wherein the cycle sequencing primer for CYT b gene consisted of oligonucleotides with the sequence:
CBI-L: 5' CCA TCC AAC ATC TCA GCA TGA TGA AA 3'
32. A method claimed in claim 1 wherein the cycle sequencing forward primer for D-Loop region consisted of oligonucleotides with the sequence:
PRO-L: 5' CTA CC 3'
33. A method claimed in claim 1 wherein the backward cycle sequencing primer for D-Loop region consisted of oligonucleotides with the sequence:
D-LOOP H: 5' CCT GAA GTA GGA ACC AGA TG 3'
34. A method as claimed in claim 1 wherein the forward primer used for cycle sequencing of ITS2 gene consisted of oligonucleotides with the sequence:
ITS 1 -F: 5' TTG TAC ACA CCG CCC GTC GC 3'
35. A method as claimed in claim 1 wherein the backward primer used for cycle sequencing of ITS2 gene consisted of oligonucleotides with the sequence:
ITS2 -R: 5' ATA TGC TTA AAT TCA GCG GG 3'
36. A method as claimed in claim 1 wherein the forward primer used for cycle sequencing of Rhodopsin gene Rod consisted of oligonucleotides with the sequence:
ROD-F: 5' CAT ATG AAT ACC CTC AGT ACT ACC 3'
37. A method as claimed in claim 1 wherein the backward primer used for cycle sequencing consisted of oligonucleotides with the sequence:
ROD-R: 5' TCT TTC CGC AGC ACA ACG TGG 3'
38. A method as claimed in claim 1 wherein the forward primer used for cycle sequencing of 12S RNA gene consisted of oligonucleotides with the sequence:
12 SA-L: 5' AAA CTG GGA TTA GAT ACC CCA CTA T 3'
39. A method as claimed in claim 1 wherein the backward primer used for cycle sequencing of 12S RNA gene consisted of oligonucleotides with the sequence:
12 SB-H: 5' AGA GTG ACG GGC GGT GTG T 3'

40. A method as claimed in claim 1 wherein the forward primer used for cycle sequencing of 16S RNA gene consisted of oligonucleotides with the sequence:
16 SAR -L: 5' CGC CTG TTT ATC AAA AAC AT 3'
41. A method as claimed in claim 1 wherein the backward primer used for cycle sequencing of 16S RNA gene consisted of oligonucleotides with the sequence:
16 SBR-H: 5' CCG GTC TGA ACT CAG ATC ACG T 3'
42. A method as claimed in claim 1 wherein the extension products of 12 S RNA gene region are purified by conventional methods.
43. A method as claimed in claim 1 wherein the extension products of 16 S gene region are purified by conventional method.
44. A method as claimed in claim 1 wherein the extension products of CYT b gene are purified by conventional method.
45. A method as claimed in claim 1 wherein the extension products of ROD gene are purified by conventional method.
46. A method as claimed in claim 1 wherein the extension products of D-Loop control region are purified by conventional method.
47. A method as claimed in claim 1 wherein the extension products of ITS2 region are purified by conventional method.
48. A method as claimed in claim 1 wherein the extension products of 12 S RNA gene region was sequenced in an automated sequencer.
49. A method as claimed in claim 1 wherein the extension products of 16 S gene region was sequenced in an automated sequencer.
50. A method as claimed in claim 1 wherein the extension products of CYT b gene was sequenced in an automated sequencer.
51. A method as claimed in claim 1 wherein the extension products of ROD gene was sequenced in an automated sequencer.
52. A method as claimed in claim 1 wherein the extension products of D-Loop control region was sequenced in an automated sequencer.
53. A method as claimed in claim 1 wherein the extension products of ITS2 region was sequenced in an automated sequencer.

54. A method as claimed in claim 1 wherein the identity of the gene 12S RNA is confirmed by Blast Email.
55. A method as claimed in claim 1 wherein the identity of the gene 16S RNA is confirmed by Blast Email.
56. A method as claimed in claim 1 wherein the identity of the gene CYT b is confirmed by Blast Email.
57. A method as claimed in claim 1 wherein the identity of the gene ROD is confirmed by Blast Email.
58. A method as claimed in claim 1 wherein the identity of the D-Loop is confirmed by Blast Email.
59. A method as claimed in claim 1 wherein the identity of the gene ITS2 is confirmed by Blast Email.
60. A method as claimed in claim 1 wherein the vector used for cloning was Bluescript KS⁺ phagemid.
61. A method as claimed in claim 1 wherein the vector used for cloning had ampicillin resistance gene for selection.
62. A method as claimed in claim 1 wherein the vector used for cloning had Lac Z gene for blue white colony selection.
63. A method as claimed in claim 1 wherein the Col E I was the origin for replication of phagemid in the absence of helper phage.
64. A method as claimed in claim 1 wherein F⁺ I⁻ origin for recovery of antisense strand of lac Z gene when a host strain containing the bluescript II phagemid.
65. A method as claimed in claim 1 wherein the host cells used for transformation were E. coli blue bacteria (Bacteria Strain XL 1 blue) XL1-Blue :- F⁺ ::Tn10,pro A⁺B⁺lacI^q (lacZ)M15/recA1endA1gyrA96(Nal^r)thi hsdR17(r_k⁻ m_k⁺)supE44relA1 lac.
66. A method as claimed in claim 1 wherein probes are containing oligonucleotide sequences are cloned Cyt b , D-Loop, ITS2 and Rod genes.
67. A method as claimed in claim 1 wherein the probes of CYT b gene is an oligonucleotide sequence named as PSL CYTL.

68. A method as claimed in claim 1 wherein the probes of ITS 2 gene is an oligonucleotide sequence named as PSL ITS 2F.
69. A method as claimed in claim 1 wherein the probes of D-Loop control region gene is an oligonucleotide sequence named as PSL PROL.
70. A method as claimed in claim 1 wherein the PCR amplified sequence of ROD gene probe is named as ROD SLMB.
71. A method as claimed in claim 1 wherein the PCR amplified sequence of D-Loop gene probe is named as D-Loop SLMB.
72. A method as claimed in claim 1 wherein the PCR amplified sequence of ITS 2 gene probe is named as ITS 2 SLMB.
73. A method as claimed in claim 1 wherein the PCR amplified sequence of Cyt b gene probe is named as Cyt L SLMB.
74. The nucleotide base sequences of PSL CYTL (748 bp) comprising :

5'

CTTNECCATT	TTGGGCGCTT	NGGCNCGCTN	CTCCNCGAGA	CTCTGCGTAN
TAATCCAANT	CNCTNCGGGC	CNCTCCCTAC	CANTNCGCTA	CACCNCAAAT
TNCAACCCNG	TTTCCTCATC	ANTCAACCAC	ATCTGTCGAA	AACNTCAACT
ACGGCTGACT	AATCCGAAAA	CATGCACGCT	AACGGTGCCT	CTTTCTTCTT
CATCTGTATT	TATCTNCNCN	TTGGANGAGG	ACTATNCTAC	GGATCCTACC
TCTACGAAGA	GACGTGAGGT	GTTGGTGTTA	TTCTTCTCCT	TCTAATAATG
ATGACTGCNT	TTGTTGGCTA	TGTGCINCCC	NGAGGACAAA	TGTCCTTTTG
AGGTGCTACT	GTCATTACAA	NCCTACTCTC	TGCTGTNCCG	TNTGTINGCG
GCNCTCTANT	TCAATGAATT	TGAGGTGGCT	TCTCCGTAAA	CACGCAACGC
TCACTCGTTT	CTTCGCNTTC	CACTTCTTGT	TCCCATTTGT	TGTCGCNGCT
ATAACCNNGG	TTCACCNAT	TTNCCGACAT	CAAACAGGCT	CTAAANCCCC
CCCGGNTTGA	CTCCATACAA	CAAAACCCTC	CACCCTATTC	NCTATAAAAC
TCTAGGTTCTG	TGCCCCGATT	GGCTTACTTC	ATGNCTATTT	CCCGNCGGA
GGGACNAAAA	TTCTGCACC	CCCTCCCCNC	AAAATAAANA	ATGTGTCTNT
CCTACCANAA	AACAACNNAN	ACGGGGTNTG	CNCTTCCATC	ATCCACN 3'

75. The nucleotide base sequences of PSLITS2F comprises :(225BP)

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5'
TCTACGATCT   ACCGGCNTTT   NNTGTGGAAA   GACGATCATG
CATTTATGTG   TGTCTTTCTA   TGGATTTGAA   CCGTGTGGTA
CGTCTTTGCG   TACTGCTTGG   AAGGCTCAAC   TTGCTTCTGT
CCTTCICTTG   CAGTCTCGCA   CTGTCTATGC   AACGTGTTCT
ACTTCGACTT   CTGTCGAAAA   ATCTTACTTT   TGACCTCAGA
TCAGACAAGA   CTACCCGCTG   AATTT          3'

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76. The nucleotide base sequences of PSL PROL comprises :(749 BP)

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5'
CCTTTTCGGN   ATAGGCCCAN   CTCAAATGAA   TTCCTTCTCT
CCTGGTCCAA   GOCCAAAGCT   TGGACGGCAG   GTTGACAATG
GTTACAAATC   GTGACAAATC   GGCTACATAA   TTGCCGATAG
CGATGTCGTC   AAACCAAGTC   AAACAATGGC   CGATGTATAT
CGGCCAAAACC   CATATATGGG   TCTGGCTGTA   GTTTGTGTTG
AGCAACGTCA   CACCAGTGTC   TGGTCAGCAT   ATAAGATGTT
GACATCTTGC   AACATCTTAC   CCACAGACAG   ACAGTTACGG
CTGCTTACGA   ANGGCGCTAG   TGTGTGGTGT   AGAAACGAAG
ATACATACGT   CAAACAGACG   CCGGTGCACT   TGAAGACACT
GTTTGAAGGT   GCCGCACTAC   TTGACAGACA   GCCCATGATG
CGCTGGACAG   TGACCAAAGC   TACNGGAGGA   CCANATGGAA
ATCCTGTGTTG   CGTTGCCGTG   GGACTCAAAGT   TGTACACTTT
TGGATGGTTG   ATCACTANAN   CCGCTGCCGG   GAGAAGCACT
CGCTCCTGGT   TCACTAATCA   GATTGAGGTT   AACCANATTG
ANGTAAACAT   CTTCAACACA   GTGTCTTTAT   GCTGGATGAA
ATTNAGCCCA   CNGGACACCA   NAAAAGAATT   NCCNCTGGTT
CTNNCGGGGG   NCCCNNNAA   CGNNNTTCC   CCTTNTCTCN
NNNGCGGNGA   AGTTNCCCCC   CCCCACTNAN   NTCTTCCTTC
AANANNTTTC   CNCCNNNAGA   GGTTTTCCTN   3'

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77. The nucleotide base sequences of ROD PSL SLMB comprises: (748 BP)

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5'
CCTGGTAGGG   TTCCCCGTCA   ACTTCTCAG   ACTGTACCTC
ACNTTCGAGC   ACAAGAAGCT   ACTAACCCCC   TTAAACTACA
TCCTGCTCAA   CCTGGCGGTC   GGAGACCTCC   TGATGGTGTA
AGGAGGGTTC   ACCACCACCA   TCTACACCTC   CATGCACGGC
TACTTCGTCC   TAGGGAAACT   GGGCTGCGCC   ATCGAAGGTT

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TCATGGCCAC	CCATGGTGGT	CAGGTCGCC	TTTGGTCCCT
GGTGGTTTTG	GCCGTGGAAA	GGTGGCTGGT	CGTCTGCAAN
CCCATCTCCA	GCTTCCGCTT	CCAGGAGTCC	CACTCCCTCA
TGGGCCTGGC	CGTGACCTGG	GTGATGGCGA	CGGCTTGTTT
TGTGCCCCC	CTGGGTCCGC	TGGTCTCGCT	ACATCCCAGA
AGGCATGCAG	TGCTCATGCG	GAATGGACTA	CTACTCTCC
GCGCCGGGCG	TCAACAATGA	ATCCTACGTN	GTGTACATGT
TCNTCANAAA	AANAATNGGA	CCNCNGGGCG	ATCATNTTGN
TANGNNAAGG	CCAGNTGNTG	NGAGCAGTCA	AGGCGGCGCG
CGCCGCCCAG	CAAGAGTCCG	AGACCACCCA	GAGGGCCGAG
AGGGAAGTCA	CCCGNATGGT	NATNANGATG	GTNATNTCNT
TCNTGGTAAG	NAGGGNGCCA	NACGCCAGCG	TGGCCTGGTG
GATCTTNQNN	AACCAGGGNG	CAGAATTAGG	CCNNGTNTTC
ATGACCCCTGC	CGGCNTTCTT	TGCCAAGA	3'

78. A method as claimed in claim 1 wherein FORWARD (L) primers of CYT b gene region for myctophid fish *Stenobrachius leucopsarus* is an oligonucleotide comprising:

5' CAA CCT CAT CTG TCG TAA AC 3'

and having the following characteristics:

- i. is a 20-mer DNA oligonucleotide (sense),
- ii. has melting temperature of 56.4 degree celcius,
- iii. has a molecular weight of 6101.0,
- iv. has no hairpin loops,
- v. has no single dimers,
- vi. has no other dimers,
- vii. has no single bulge loops or internal loops, and
- viii. has no palindromes.

79. A method as claimed in claim 1 wherein BACKWARD (H) primer of CYT b gene region for myctophid fish *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising :

5' GCT CGG GCT GCT GGA ATC TT 3'

and having the following characteristics:

- i. is a 20-mer DNA

- ii. is an antisense oligonucleotide
 - iii. has a melting point of 70.8 degree celcius.
 - iv. has a molecular weight of 6220.1.
 - v. has no hairpin loops, no single bulge loops, no other internal loops, no single internal loops, no other bulge loops or palindromes.
 - vi. no single dimers or other dimers.
80. A method as claimed in claim 1 wherein forward primer of ITS2 F gene region for myctophid fish *Stenobranchius leucopsarus* (SLMB) is an oligonucleotide comprising:
- 5' ACT TGA CTG ACC TTC TTA CT 3'
- and having the following characteristics:
- i. is a 20-mer sense oligonucleotide,
 - ii. has a melting point of 51.3 degree celcius,
 - iii. has a molecular weight of 6098.0,
 - iv. has no palindromes, loops and dimers,
81. A method as claimed in claim 1 wherein forward primer of ITS2 H gene region for myctophid fish *Stenobranchius leucopsarus* (SLMB) is an oligonucleotide comprising :
- 5' ATA CTC TGC GGA CAT ACT TGA CTG 3'
- and having the following characteristics:
- i. is a 24-mer antisense oligonucleotide,
 - ii. has a melting point of 65.4 degree celcius.
 - iii. has a molecular weight of 7407.9.
 - iv. has no palindromes, loops and dimers.
82. A method as claimed in claim 1 wherein forward primer of pro-L for myctophid fish *Stenobranchius leucopsarus* (SLMB) is an oligonucleotide comprising:
- 5' CAG TCT CGT CAA ACC AAG TCA AAC 3'
- and having the following characteristics:

- i. is a 24-mer sense oligonucleotide
- ii. has a melting point of 67.8 degree celcius.
- iii. has a molecular weight of 7354.9.
- iv. has no palindromes, loops and dimers.

83. A method as claimed in claim 1 wherein backward primer for Dloop for mitochondrial control region (dloop H) gene region for myctophid fish *Stenobranchius leucopsarus* is an oligonucleotide comprising :

5' ATA ATC ATC CAG CAT AAA CAC AC 3'

and having the following characteristics:

- i. is a 23-mer antisense oligonucleotide,
- ii. has a melting point of 61.2 degree celcius.
- iii. has a molecular weight of 7033.7.
- iv. has no palindromes, loops and dimers.

84. A method as claimed in claim 1 wherein the FORWARD primer (ROD- L) for Rhodopsin gene region of myctophid fish *Stenobranchius leucopsarus* is an oligonucleotide comprising:

5' CCT GGT AGA GTT CGC CGT CA 3'

and having the following characteristics:

- i. is a 20-mer sense oligonucleotide
- ii. has a melting point of 67.4 degree celcius.
- iii. has a molecular weight of 6189.0.
- iv. has no palindromes, loops and dimers.

85. A method as claimed in claim 1 wherein the backward primer (ROD- H) for Rhodopsin gene region of myctophid fish *Stenobranchius leucopsarus* is an oligonucleotide comprising:

5' CGT GTT CCT TAT CAT TGT GCC T 3'

and having the following characteristics:

- i. is a 22-mer antisense oligonucleotide

- ii. has a melting point of 66.4 degree celcius.
 - iii. has a molecular weight of 6738.4.
 - iv. has no palindromes, loops and dimers.
86. A method as claimed in claim 1 wherein the forward primer of 16S-L of the myctophid fish *Lampanyctus regalis* is an oligonucleotide comprising:
5' CAC CAG CCA AGT ATG TTT CTC 3'
and having the following characteristics:
- i. is a 21-mer sense oligonucleotide
 - ii. has a melting point of 61.5 degree celcius.
 - iii. has a molecular weight of 6421.4.
 - iv. has no palindromes, loops and dimers.
87. A method as claimed in claim 1 wherein the backward primer of 16s rRNA of myctophid fish *Lampanyctus regalis* is an oligonucleotide comprising:
5' TCG TAG TTC AGC AGT CAG 3'
and having the following characteristics:
- i. is a 18-mer antisense oligonucleotide
 - ii. has a melting point of 51.2 degree celcius.
 - iii. has a molecular weight of 5594.7.
 - iv. has no palindromes, hairpin loops and dimers.
88. A method as claimed in claim 1 wherein the forward primer 16S-L of myctophid fish *Lampanyctus regalis* is an oligonucleotide comprising:
5' CTA TTC GCC TCG CTC AGA C 3'
and having the following characteristics:
- i. is a 19-mer sense oligonucleotide
 - ii. has a melting point of 62.1 degree celcius.
 - iii. has a molecular weight of 5779.8.
 - iv. has no palindromes, hairpin loops and dimers.

89. A method as claimed in claim 1 wherein a primer 12S-H for *Lampanyctus regalis* (LRMB) is an oligonucleotide comprising:
5' GCC TCC ATC ATC CCT CAC CTT AC 3'
and having the following characteristics:
i. is a 23-mer antisense oligonucleotide
ii. has a melting point of 70.8 degree celcius.
iii. has a molecular weight of 6895.5
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
90. A method as claimed in claim 1 wherein the primer 12S-L for *Lampanyctus regalis* (LRMB) is an oligonucleotide comprising:
5' CTA TTC GCC TCG CTC AGA C 3'
and having the following characteristics:
i. is a 19-mer sense oligonucleotide
ii. has a melting point of 62.1 degree celcius.
iii. has a molecular weight of 5779.8
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
91. A method as claimed in claim 1 wherein 16S-L forward primer for *Diaphus theta* (DTMB) is an oligonucleotide comprising:
5' AAA TCC GCC CTT ATG TGT GTT C 3'
and having the following characteristics:
i. is a 22-mer sense oligonucleotide
ii. has a melting point of 67.9 degree celcius.
iii. has a molecular weight of 6756.4
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

92. A method as claimed in claim 1 wherein 16S-H backward primer for *Diaphus theta* (DTMB) is an oligonucleotide comprising:
5' CTC CGT CCG TCT CGC CTC TG 3'
and having the following characteristics:
i. is a 20-mer antisense oligonucleotide
ii. has a melting point of 71.7 degree celcius.
iii. has a molecular weight of 6052.0
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
93. A method as claimed in claim 1 wherein 12S-H forward primer for *Diaphus theta* (DTMB) is an oligonucleotide comprising:
5' CAT CGG CTT GCT CTA TTC CTT G 3'
and having the following characteristics:
i. is a 22-mer antisense oligonucleotide
ii. has a melting point of 68.8 degree celcius.
iii. has a molecular weight of 6723.4
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
94. A method as claimed in claim 1 wherein 12S-L forward primer for *Diaphus theta* (DTMB) is an oligonucleotide comprising:
5' TCT ATC GGC GGC GTA TCA C 3'
and having the following characteristics:
i. is a 19-mer sense oligonucleotide
ii. has a melting point of 65.8 degree celcius.
iii. has a molecular weight of 5859.8
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

95. A method as claimed in claim 1 wherein 16S-H primer for *Tarletonbeania crenularis* (TCMB) is an oligonucleotide comprising:
5' GGC GAT TCT ACG GCA CGG GCG 3'
and having the following characteristics:
i. is a 21-mer antisense oligonucleotide
ii. has a melting point of 80.4 degree celcius.
iii. has a molecular weight of 6568.3
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
96. A method as claimed in claim 1 wherein 16S-L forward primer for *Tarletonbeania crenularis* (TCMB) is an oligonucleotide comprising:
5' AAA CTG GTC CTC AAC TAT GTC A 3'
and having the following characteristics:
i. is a 22-mer sense oligonucleotide
ii. has a melting point of 60.7 degree celcius.
iii. has a molecular weight of 6758.5
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
97. A method as claimed in claim 1 wherein 16S-H backward primer for *Tarletonbeania crenularis* (TCMB) is an oligonucleotide comprising:
5' GGC GAT TCT ACG GCA CGG GCG 3'
and having the following characteristics:
i. is a 21-mer antisense oligonucleotide
ii. has a melting point of 80.4 degree celcius.
iii. has a molecular weight of 6568.3
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

98. A method as claimed in claim 1 wherein 12S-H backward primer for *Tarletonbeania crenularis* (TCMB) is an oligonucleotide comprising:
5' CCG ATT CAG CCA CGA TTC CCT C 3'
and having the following characteristics:
i. is a 22-mer antisense oligonucleotide
ii. has a melting point of 74.6 degree celcius.
iii. has a molecular weight of 6671.4
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
99. A method as claimed in claim 1 wherein 12S-L forward primer for *Tarletonbeania crenularis* (TCMB) is an oligonucleotide comprising:
5' CCT AAA GCC CAG ATA ACT ACA 3'
i. is a 21-mer sense oligonucleotide
ii. has a melting point of 59.2 degree celcius.
iii. has a molecular weight of 6432.3
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
100. A method as claimed in claim 1 wherein 16S-H backward primer for *Protomyctophum crockeri* (PCMB) is an oligonucleotide comprising:
5' CGT GTT CTG ATG ATG ATG TGC T 3'
i. is a 22-mer antisense oligonucleotide
ii. has a melting point of 64.7 degree celcius.
iii. has a molecular weight of 6867.5
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
101. A method as claimed in claim 1 wherein 16S-L forward primer for *Protomyctophum crockeri* (PCMB) is an oligonucleotide comprising:
5' ATT CCT TCC TCT TAG TAT G 3'

- i. is a 19-mer sense oligonucleotide
 - ii. has a melting point of 49.5 degree celcius.
 - iii. has a molecular weight of 5799.8
 - iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
102. A method as claimed in claim 1 wherein 12S-H backward primer for *Protomyctophum crockeri* (PCMB) is an oligonucleotide comprising:
- 5' GCT GAA CTT ACT ATG CCC TAC T 3'
- i. is a 22-mer antisense oligonucleotide
 - ii. has a melting point of 60.3 degree celcius.
 - iii. has a molecular weight of 6725.4
 - iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
103. A method as claimed in claim 1 wherein 12S-L forward primer for *Protomyctophum crockeri* (PCMB) is an oligonucleotide comprising:
- 5' CCG ATT GAC GCC GAA CTA TG 3'
- i. is a 20-mer sense oligonucleotide
 - ii. has a melting point of 68.1 degree celcius.
 - iii. has a molecular weight of 6182.1
 - iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
104. A method as claimed in claim 1 wherein 16S-H backward primer for *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising:
- 5' TAC GCA TAA CGG CTC TGG 3'
- i. is a 18-mer DNA oligonucleotide (Antisense)
 - ii. has a melting point of 61.4 degree celcius.
 - iii. has a molecular weight of 5579.7

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

105. A method as claimed in claim 1 wherein 16S-L forward primer for *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising:

5' CTA CTA CAC CTC AAC TAC ATC T 3'

- i. is a 22-mer sense oligonucleotide
- ii. has a melting point of 52.4 degree celcius.
- iii. has a molecular weight of 6638.4
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

106. A method as claimed in claim 1 wherein 12S-H forward primer for *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising:

5' CCC ACT CAC TGC TAA CTC C 3'

- i. is a 19-mer sense oligonucleotide
- ii. has a melting point of 58.4 degree celcius.
- iii. has a molecular weight of 5708.8
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

107. A method as claimed in claim 1 wherein 12S-L forward primer for *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising:

5' GGC TAA CTA CAA TCA TCT GCT 3'

- i. is a 21-mer sense oligonucleotide
- ii. has a melting point of 58.5 degree celcius.
- iii. has a molecular weight of 6445.2
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.